Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE PRODUCTION OF HYDROCYANIC ACID BY BACILLUS PYOCYANEUS

F. ARTHUR PATTY

From the Department of Bacteriology, University of Kansas, Lawrence

Emerson, Bailey, and Cady noted the formation of hydrocyanic acid in decaying protein.[1] Clawson and Young in a series of qualitative experiments reported that a number of different strains of bacteria produce hydrocyanic acid.[2] In addition to these reports Kendall, Day and Walker included B. pyocyaneus with other bacteria in some of their studies on bacterial metabolism.[3]

The purpose of this work was (1) to determine the optimum reaction for the production of HCN by strains of B. pyocyaneus; (2) to devise a quantitative method of determining the amount of HCN produced; (3) to determine the effect of anaerobic growth; (4) to determine whether HCN is produced by an extracellular enzyme; (5) to determine any correlation between the amount of HCN, pigmentation, and rapidity of liquefaction of gelatin; (6) to determine relative amounts of HCN produced in gelatin, broth, eggwhite, whole egg, and synthetic broth; (7) to note the effect on the amount of HCN of non-HCN-producing organisms; (8) to see if B. pyocyaneus will produce HCN in the animal body after death, as this might be of some medicolegal importance.

Nine strains of B. pyocyaneus were used in the experiments, all of which were known cultures from reputable sources, as follows: No. 68 from the American Museum; 184 from the University of Kansas; 174, 175, 176, 177, 178, and 180 from the University of Chicago and Rush Medical College, and 179 from the University of Michigan.

The determination of hydrogen-ion concentration of mediums was made by the colorimetric method with buffers according to Cole.[4]

Qualitative tests for HCN were made by the Shoenbein or guaiac copper method, which is open to many objections and can be used only as a preliminary test. The quantitative estimation was carried on as follows: The organisms were grown in medium contained in 250 c c Erlenmeyer flasks, fitted with 2 hole stoppers, and air, filtered through soda lime and cotton, was aerated through the flasks into 4% KOH solution. At the end of 7 days the KOH solution was added to the medium, the whole made acid with sulphuric acid and distilled into 4% KOH. One c c yellow ammonium sulphide was added to

Received for publication, Feb. 28, 1921.

[1] Practical Physiologic Chemistry, 1919, p. 24.

[2] Ibid., p. 419.

[3] J. Am. Chem. Soc., 1914, 36, p. 1937.

[4] Biol. Chem., 1919, 40, p. 243.

the distillate, and the whole evaporated to dryness on a water bath. The residue was extracted with three 10 c c portions of acetone, and the acetone evaporated off and water added. Fifteen drops of 5% ferric chloride solution were added to the potassium sulphocyanate solution, and the color compared to that produced by a standardized solution of KSCN: 1 c c equals 0.01 mg. HCN.

Anaerobic conditions were obtained by growing the organisms in bottles which were sealed into a large jar containing sodium hydroxide solution and pyrogallol.

In order to find whether HCN is produced by an extracellular or intracellular enzyme, broth culture of B. pyocyaneus was filtered through a Berkefeld filter and the filtrate allowed to stand in gelatin for 7 days, when the HCN content was determined.

To learn whether HCN is produced in the animal body, 5 c c of an 18-hour old broth culture of B. pyocyaneus were injected intravenously into each of 4 rabbits, which were killed in a few hours. The liver, spleen, and stomach of each were distilled from acid solution into 4% KOH and subsequently tested for HCN. The rabbits were allowed to lie in an ice box at 10 C. until the distillations were made.

TABLE 1

Variation of HCN Due to Hydrogen-Ion Concentration

Strain	20% Whole Egg Broth, P_H 5.6, Percentage	20% Egg White Broth, P_H 6.8, Percentage	Gelatin, P_H 5.8, Percentage	Gelatin, P_H 6.8, Percentage	Synthetic Broth, P_H 7, Percentage
68	0.127	0.0043	0.00686	0.00183	0.0125
184	0.113	0.0157	0.00688	0.00309	0.0145
174	0.0069	0.0041	0.00064	0.0028	0.050
175	0.0577	0.0098	0.0016	0.0101	0.0135
176	0.0115	0.0059	0.00141	0.00064	0.040
177	0.173	0.0075	0.0081	0.0114	0.0085
179	0.0052	0.0046	0.000686	0.00081	0.0035
180	0.0298	0.0051	0.00297	0.00064	0.030
178	0.0035	0.0041	0.00066	0.000686	0.075

The first distillation was made 12 hours, the second 24 hours, the third 48 hours, and the fourth, 7 days after death. Three controls were carried along simultaneously. On one the distillation was made at death, and on the other two, 7 days after death, one having been kept intact at 10 C. and the other operated on, and the liver, spleen, and stomach removed under sterile conditions and preserved at 10 C.

The medium used in this work was made according to formulae in "Standard Methods of Water Analysis, 1917." The synthetic broth is essentially that used in the methyl red test.

Variation of HCN due to hydrogen-ion concentration is given in table 1, which gives HCN % of total nitrogen.

Most strains yield more HCN at P_H 5.6 to 5.8. Cultures 174 and 178, strong acid producers, appear to produce HCN better at lower hydrogen-ion concentration, while 68 and 180, unusually strong alkali producers, show a decrease of HCN in medium of lower hydrogen-ion concentration.

The presence of oxygen plays an important part in HCN production as shown in table 2.

Little HCN is produced anaerobically and the amount is practically constant for the change in P_H. A plain broth culture of B. pyocyaneus, preserved anaerobically for 16 months, was found to be alive and a good green pigment producer when transferred to agar, but no HCN was found in the broth.

TABLE 2

Results with Strain 184 Both in Mmg. of HCN and HCN Percentage of Total Nitrogen

	Gelatin, P_H 5.8		Gelatin, P_H 7.8	
Aerobic	0.335 mmg.	0.00773%	0.100 mmg.	0.00228%
Anaerobic	0.0321 mmg.	0.000739%	0.0322 mmg.	0.000740%

In the gelatin, to which 1 c c of sterile filtrate from a pyocyaneus broth culture was added, no HCN was produced although the gelatin was liquefied.

There is a certain correlation between the amount of HCN, pigmentation and liquefaction, as shown in table 3.

TABLE 3

Correlation Between Amount of HCN, Pigmentation and Liquefaction

Strain	Pigmentation	Gelatin Liquefaction	Mg. HCN in Gelatin, P_H 5.8
68	Dark green	Good	0.30
184	Dark green	Good	0.335
174	Brown	Slow	0.028
175	Green	Good	0.070
176	Green	Good	0.050
177	Light green	Good	0.350
178	Brown	Slow	0.029
179	Green	Good	0.030
180	Dark green	Good	0.130
Blank	....	......	0.028

Strains 174 and 178, brown pigment producers, were slow in liquefying gelatin and at the end of 7 days the cool gelatin cultures poured from the flasks with difficulty, while all the others were a thin liquid. The two organisms did not produce a measureable amount of HCN. However, pigmentation and HCN production are nevertheless independent functions, for lowering the HCN formation by lowering the hydrogen-ion concentration does not diminish pigment production.

TABLE 4

RELATIVE PERCENTAGES OF HCN PRODUCED IN VARIOUS MEDIUMS EXPRESSED AS HCN% OF TOTAL NITROGEN

Strain	20% Egg White Broth, Percentage	20% Whole Egg Broth, Percentage	Gelatin, Percentage	Synthetic Broth, Percentage
68	0.0043	0.1270	0.00686	0.0125
184	0.0157	0.1130	0.00688	0.0145
174	0.0041	0.0069	0.0064	0.0500
175	0.0098	0.0577	0.0016	0.0135
176	0.0059	0.0115	0.00114	0.0400
177	0.0075	0.1730	0.0081	0.0085
178	0.0041	0.0035	0.00066	0.0750
179	0.0046	0.0052	0.000686	0.0035
180	0.0051	0.0298	0.00297	0.030
Blank	0.0041	0.0035	0.00064	0.0035

The quantity of HCN ranges from a mere qualitative test to 0.173% of the total available nitrogen. Whole egg broth 20% seems to be the most favorable medium for HCN formation and gelatin is about the least productive, due in part at least to the fact that not all the gelatin is immediately acted on.

TABLE 5

THE EFFECT ON HCN PRODUCTION OF A NON-HCN-PRODUCING COMBINATION IN MG. OF HCN.

Strain	Gelatin Original Culture, Mg.	Gelatin, Culture Recovered from Egg, Mg.
68	0.300	0.260
184	0.335	0.270
174	0.028	0.026
175	0.070	0.100
176	0.050	0.030
177	0.350	0.250
178	0.029	0.050
179	0.030	0.028
180	0.130	0.090
Control	0.028	0.028

Growth in egg white does not seem to increase the HCN producing power of the bacillus, as has been stated;[1] in fact most strains produce HCN better in gelatin before growth in egg white than after. The contaminating organisms introduced in egg white were gram-negative rods but not HCN producers.

The results of animal inoculation (table 6) are interesting for they show an increasing amount of HCN in the bodies of the inoculated rabbits.

TABLE 6
RESULTS OF ANIMAL INOCULATION
Inoculated Rabbits

1. Distillation, 12 hours after death, 0.010 mg. HCN
2. Distillation, 24 hours after death, 0.016 mg. HCN
3. Distillation, 48 hours after death, 0.020 mg. HCN
4. Distillation, 7 days after death, 0.085 mg. HCN

Controls

1. Distillation at death.....................0.007 mg. HCN
2. Distillation after 7 days in body...0.050 mg. HCN
3. Distillation after 7 days sterile.....0.045 mg. HCN

The 5 c c broth used for inoculation, contained 0.003 mg. HCN. It is evident from the data that a measureable quantity of HCN is produced in the viscera of a rabbit after death. Since B. pyocyaneus is frequently found in the human body, several strains having been recovered here from feces of influenza patients, it is reasonable to believe that in postmortem examination sufficient HCN may be produced by B. pyocyaneus to give a positive test for cyanide.

SUMMARY

The optimum reaction for the production of HCN by most strains of B. pyocyaneus is P_H 5.4 to 5.8.

The sulphocyanate colorimetric method of estimating minute quantities of HCN is a satisfactory one, but control tests must always be made, because in protein medium there is sufficient sulphocyanate formed on distillation to give a positive reaction.

Oxygen is necessary for the production of HCN by B. pyocyaneus.

HCN is not produced by a filtrable extracellular enzyme.

Pigmentation, gelatin liquefaction, and HCN production, though independent functions, show a close relationship.

Whole egg broth is the most favorable medium for HCN production, but synthetic medium is second, indicating that a favorable synthetic medium may be devised.

Non-HCN-producing contaminating organisms appear to be slightly inhibitive of HCN formation.

B. pyocyaneus does produce a measureable amount of HCN in the animal body, which may be of some medicolegal importance.

There is a marked variation among the different strains of B. pyocyaneus as to the amount of HCN production.